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Role of the *PROS1* gene in thrombosis: lessons and controversies

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Functions of protein S

The *PROS1* gene codes for the vitamin-K-dependent plasma protein, protein S (PS), an essential anticoagulant and multifunctional protein. The association of PS with thrombosis [1] is well established, but the particular anticoagulant activity or activities of PS that are compromised in thrombosis are not entirely clear and the effects of PS deficiency on more recently discovered functions of PS in inflammation, apoptosis and receptor activation are unknown. Lessons can be learned from the phenotypes of reported mutations and polymorphisms in the *PROS1* gene, but many questions and controversies remain. For greater depth, the reader is referred to extensive databases of PS mutations [2,3], recent reviews [4, 5] and molecular models that rationalize PS mutations [6,7].

PS cofactor activity for activated protein C

The best-known anticoagulant function of PS is to serve as a cofactor to activated protein C (APC) in the proteolytic inactivation of potent coagulation factors (F) Va and VIIIa [8]. When functional assays are performed in the clinic, this is the activity that is measured but, more often, activity is inferred from antigen measurements. Total PS antigen measures free PS (normally 40% of plasma PS), as well as PS in complex with C4b-binding protein (C4BP). PS cofactor activity for APC is almost exclusively exhibited by free PS, thus, assays for free PS are more indicative of deficient APC cofactor activity of PS. Owing to the high affinity of PS for C4BP, in most cases of PS deficiency, free PS is diminished to a greater extent than total PS. Thus, type I deficiency is the deficiency of both total and free PS, whereas type III deficiency is the deficiency of free but not total PS, and there has been a search for differences in mutations and phenotypes between types I and III. Rare type II deficiencies are those with a functional but not a quantitative insufficiency. In assays for APC cofactor activity, false positives for PS deficiency are occasionally obtained in FV Leiden-containing plasma [9,10]. This may be due to inefficient PS cofactor activity in synergy with FV Leiden [11].

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Direct anticoagulant activity of PS: is it physiologically important?

Protein S also has direct anticoagulant activity (PS-direct), independent of APC; however, mechanisms underlying this activity are controversial. PS inhibits the generation of thrombin by binding and inhibiting FXa and FVa in the prothrombinase complex [12–14]. By contrast, it was reported that the sole mechanism of PS-direct is that of a cofactor to tissue-factor pathway inhibitor (TFPI) during inhibition of FVIIa/tissue factor [15]. However, the latter cannot be the only mode of PS-direct, since antibodies to TFPI do not negate PS inhibition of prothrombinase in purified systems (ruling out TFPI contamination) or in prothrombinase-based assays for PS-direct in plasma [12,16]. Furthermore, TFPI is a poor inhibitor of prothrombinase. We believe that some conflicting results from experiments using purified systems were due to the previously unrecognized requirement for a Zn^{2+} ion in PS for PS-direct (but not for APC cofactor activity) and loss of Zn^{2+} during some purification procedures [17].

By whatever mechanism(s), there is evidence that PS-direct is an important activity. First, PS was antithrombotic in a baboon thrombosis model, even in the presence of an antibody that blocks protein C activation [18]. Second, some homozygous/double heterozygous PS-deficient infants suffer fatal thrombosis after birth unless given an infused source of PS. The infused PS immediately forms complexes with C4BP in the blood. These complexes are nearly devoid of APC cofactor activity [19] but have PS-direct [13,20]; yet, infused PS is protective in such infants, probably via PS-direct. Third, many heterozygous PS-deficient individuals have almost no free PS, yet their phenotype is not as severe as that of most untreated homozygous PS-deficient infants, suggesting that their PS–C4BP complexes are protective, in part, via PS-direct. Fourth, individuals with PS Heerlen (~0.5% of Caucasians) are deficient in free PS, yet they have no increased risk of thrombosis [21]. Their isolated PS–C4BP complexes are more active in inhibiting prothrombinase than normal complexes and their plasma has more PS-direct activity than expected [20]. Fifth, there were a number of reports that PS deficiency was associated with ischemic stroke at young ages [22], but protein C deficiency has not been associated with stroke, suggesting an APC-independent role for PS. Finally, at least one type II (qualitative) PS-deficient individual has a mutation in a C-terminal domain of PS [2], a domain not very important for APC cofactor activity but reported to exhibit PS-direct via FVa binding [23].

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Only one study measured PS-direct in a patient group, those with the prothrombin G20210A mutation [24]. In these patients, prothrombin was elevated (123% of normal) and PS-direct was decreased, while PS antigen level was normal. This is consistent with reports that PS and prothrombin compete for binding to the FVa heavy chain [12], but it would be interesting to learn whether APC cofactor activity in these patients is affected, since prothrombin was also reported to interfere with the cofactor activity of PS [25]. In most cases of PS deficiency, we expect both APC cofactor activity and PS-direct to be impaired, but it would be intriguing to discover cases of low PS-direct when APC cofactor activity and antigen are normal.

Association of PS deficiency with venous thrombosis

Venous thrombosis has been associated with inherited PS deficiency in 2–15% of cases, depending on nationality [2]. In large asymptomatic populations, the incidence of PS deficiency varied from 0.5 to 1.5% [4]. From kindred data, it was estimated that heterozygous PS deficiency increases the risk of venous thrombosis ten- to 20-fold [3]. Venous thrombosis is often a multifactorial condition, thus, PS deficiency is discovered in up to 30% of symptomatic

patients with FV Leiden [2] and in some symptomatic patients with other thrombophilic defects.

Acquired PS deficiency has also been frequently reported to be associated with thrombosis in cases such as septicemia, HIV and measles. The increased risk of thrombosis due to pregnancy and oral contraceptive use may be partly due to decreased PS. Indeed, PS is also significantly lower in women than in men, suggesting that PS is a hormonally sensitive coagulation factor; this merits exploration.

Thrombosis cases usually include deep vein thrombosis, pulmonary embolism or thrombophlebitis. Yet, almost weekly, a new case report appears of PS deficiency in a patient with thrombosis at an unusual site, such as in a portal, mesenteric, sagittal sinus or cerebral sinus vein. In one family, several affected individuals suffered from dorsal foot lesions [26]. These unusual cases also suggest a different role for PS than for protein C, although a PS hereditary defect was not often established. Hopefully, future cases will be examined for familial defects and mutations.

Diagnosis of PS inherited deficiency is of value to affected family members, who may benefit from the information by avoidance of compounding thrombosis risk factors. However, it has been pointed out that thrombophilia testing presently makes no difference in how a venous thrombosis patient is treated [27].

Arterial thrombosis: is there an association with PS deficiency?

There have been only a few reports of PS deficiency in patients with myocardial infarction. PS deficiency was associated with ischemic stroke at a young age in several small studies [22] and in many case reports, again suggesting an unusual phenotype for deficiency of a natural anticoagulant protein. However, there were few instances where hereditary deficiency was established or mutations were sought. This is an area worthy of further study.

Types of PS mutations: are there genetic modifiers?

Nearly 300 mutations in the *PROS1* gene have been reported, spread over all exons, the promoter region and several introns [2]. The majority are missense mutations, but all other types of mutations have been reported. In most cases, there is little/no contribution of mutant PS to total plasma PS, either due to poor secretion or rapid clearance, resulting in a quantitative deficiency. This suggests that most regions of PS at a minimum contribute to molecular stability. No more than 12 type II (qualitative)-deficient individuals were reported and only one of these had a mutation in the C-terminal sex hormone-binding globulin-like region. We speculate that other expressed mutations in the C-terminal domains may have been missed because investigators typically assay for APC cofactor activity of PS, which relies mostly on the N-terminal domains of PS, while PS-direct relies at least in part on C-terminal domains [23].

Most PS-deficient individuals are heterozygous for a PS gene defect. Nine individuals were homozygous or double heterozygous and presented as neonates with life-threatening purpura fulminans, requiring lifelong treatment [28]. Surprisingly, a few such severely deficient individuals did not experience thrombosis until 10–20 years of age [26]. This suggests that genetic modifiers exist that compensate for the risk of thrombosis caused by low PS levels. In most type III individuals (deficient in free, not total PS), no mutation is found [29], suggesting that genetic modifiers affect PS levels. In support of this, a quantitative trait locus that influences free PS levels was located on chromosome 1q [30]. Additionally, in a large kindred, levels of PS correlated strongly with levels of prothrombin, FVII, FIX and FX, suggesting a common genetic modifier [31]. Additional informative studies along these lines are anticipated.

> "…evidence mounts for direct anticoagulant activity protein S, although mechanisms are controversial."

Some mutations in PS are considered neutral, or could possibly be considered modifying mutations. It is controversial whether the relatively common Pro626Pro, nt2698 or intron-K polymorphisms are neutral. The Heerlen mutation S460P (one glycosylation site defective; as mentioned previously) appears neutral, even though it results in low free PS levels; a type I-deficient individual homozygous for this mutation was asymptomatic in early adulthood [32]. Aside from these polymorphisms, other mutations have been reported in only one family or occur rarely. An exception is the Lys155Glu mutation found in the Japanese population, which leads to a type II (qualitative) PS deficiency [33].

Broad conclusion

We conclude that, in addition to PS cofactor activity for APC, through *in vivo* and *in vitro* studies, evidence mounts for direct anticoagulant activity PS, although mechanisms are controversial. Besides typical venous thrombosis, PS deficiency is associated with thrombosis at unusual sites and may be associated with stroke. Evidence from *PROS1* mutations indicates that all regions of PS contribute significantly to molecular stability, that the N-terminal region is largely responsible for APC cofactor activity and that genetic modifiers influence PS levels in the absence of *PROS1* gene mutations.

A mouse PS gene knockout was generated and studies are underway (Burstyn-Cohen, Heeb & Lemke, Unpublished Data). This will shed new light on anticoagulant roles of PS and PS roles in inflammation and apoptosis.

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Biography

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